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SHORT NOTES

Effects of temperature and photoperiod on germination and germ tube development of dikaryotic stages of *Pileolaria terebinthi*

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Summary. The germination and germ tube development of urediospores, aeciospores and teliospores of *Pileolaria terebinthi*, the causal agent of *Pistacia* spp. rust, were investigated under different temperature and light conditions. The urediospores and aeciospores germinated in a range of 10–30°C, the optimum being 25–30°C. Both spore types died after 24 h exposure at 35°C. Incubation at -5°C for 24 h caused a significant decrease in spore germinability and germ tube growth. A significant increase in germination and germ tube growth was observed in the dark. Teliospores germinated well in a range of 15–30°C. Differentiation of basidia occurred at 20–25°C. Optimum temperature for teliospore germination and basidium differentiation was found to be 20°C. The maximum differentiation of basidia was observed in short day exposures (0.5 h light/23.5 h dark). No basidia differentiated under continuous fluorescent illumination.

Key words: pistachio rust, temperature, light, spore germination.

Introduction

Pileolaria terebinthi (DC.) Cast. is the causal agent of beneh rust, better known as pistachio rust. Beneh (*Pistacia mutica* Fisch. et Mey, Anacardiaceae) is an important forest tree in Iran, where it occupies an area of about 2.5–3 million ha (Padulosi *et al.*, 1995). Few studies have been carried out on the biology of this rust (Hamzehzarghani, 2000; Hamzehzarghani and Banihashemi, 2001). Some other reports deal only with its presence in different countries (Guyot, 1951; Assaweh, 1969; Bremer *et al.*, 1974; Dinc and Turan, 1975; Corazza and Avanzato, 1985; Corazza and Avanzato, 1986; Isikov, 1988; Bhardwaj, 1992; Grigorriu,1992; Chitzanidis, 1995). In the present study the effects of temperature and photoperiod on the germination of the dikaryotic stages of the fungus were determined.

Materials and methods

Spores (aeciospores, urediospores and teliospores) were collected from the beneh forests at Arsanjan and Firoozabad, in Fars province, Iran, in 1998 and 1999. Aeciospores and urediospores were collected from April to June, teliospores in

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September. Spores from the same leaf samples were combined to make single spore lots, which were placed in 10 ml glass vials, labelled, and maintained at 4°C in a dessicator containing $CaCl_2$. When needed, vials of spores were removed from the dessicator, rehydrated in a dew chamber for 1–2 days, suspended in sterile distilled water at a concentration of about 10^6 spores ml^{-1} and dispersed in Petri dishes filled with 2% water agar.

Percent germination of spores and germ tube growth (10 replicates) were determined under a Nikon YS2-T compound microscope at ×100 magnification following 4 h (for spore germinability) and 24 h (for germ tube growth) incubation at various temperatures (10, 15, 20, 25 and 30°C) and photoperiods (ultra short days, continuous dark, continuous light), with fluorescent illumination at 2000 lux.

To allow natural weathering of teliospores, leaves bearing telia were placed under a wirescreen frame on the floor of the forest at sites protected from direct sunlight. Teliospores were checked for germination in a drop of sterile distilled water on glass slides, and incubated in the dark in a miniature dew chamber at room temperature (20–25°C), according to Thompson (1950) and Yamaoka *et al*. (1994). Germination of the dormant teliospores stored at 4°C in the dark and in dry conditions was stimulated in several ways, by:

- soaking in running tap water for 1–3 weeks (Jennings *et al*., 1989);
- floating on distilled water at 15°C for 24–72 h (Anikster, 1986);
- heat shock (30±1°C for 4 days) (Gold and Mendgen, 1983);
- natural weathering (Thompson, 1950).

In order to reduce bacterial contamination, 40 mg l^{-1} chloramphenicol was added to the water and water agar.

Data analysis and mean comparisons were carried out in completely randomized or factorial designs and by Duncan's multiple range test respectively.

Results

Optimum temperatures for aeciospore and urediospore germination were 25–30°C (Fig. 1) and for their germ tube elongation 25°C (P<0.01) (Fig. 2). Urediospores failed to produce normal germ tubes at higher temperatures (>25°C) and germ tube growth rates decreased above 25°C (Fig. 2). Urediospores and aeciospores died when exposed at 35°C for 24 h. Germination rate and germ tube elongation decreased markedly after exposure for 24 h at -5°C, compared to controls (P<0.01).

Continuous light had a significant suppressive effect on the rate of spore germination and germ

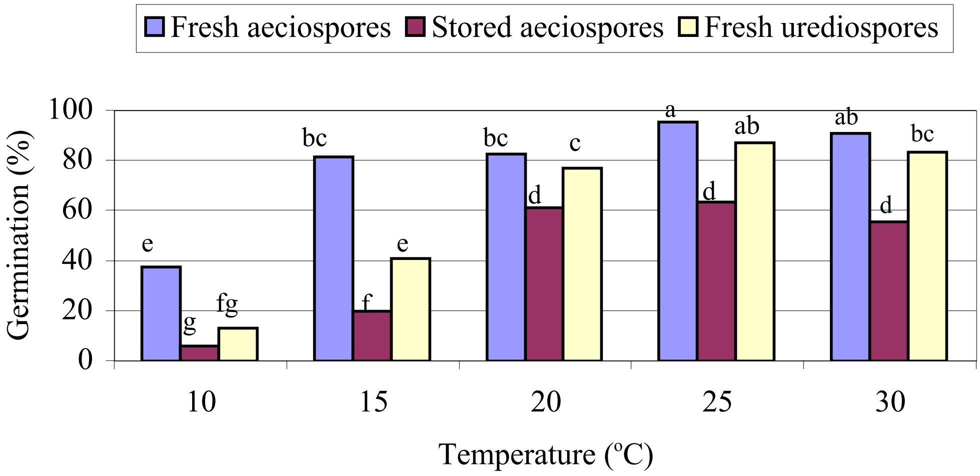

Fig. 1. Effect of temperature on percent germination of fresh and stored aeciospores and of fresh urediospores of *Pileolaria terebinthi*. Bars with the same letter(s) are not significantly different (P=0.01).

tube elongation, compared to continuous dark (P<0.01). Fresh aeciospores lost their germinability more quickly than fresh urediospores (Fig. 1, 2).

Attempts to induce teliospore germination by soaking in running tap water for even 3 weeks were unsuccessful. Four days of heat shock (at 30±1°C) increased the mortality of teliospores stored at 4°C for at least one month. The most successful treatment for teliospores germination was floating on distilled water at 15°C for 48 h. Microscope examination revealed that in all experiments teliospores became vacuolated within 2–3 days after incubation and began to germinate between 2–5 days after short day exposure at 20°C. Optimum temperature for teliospore germination and basidium differentiation was 20°C (Fig. 3). The most effective

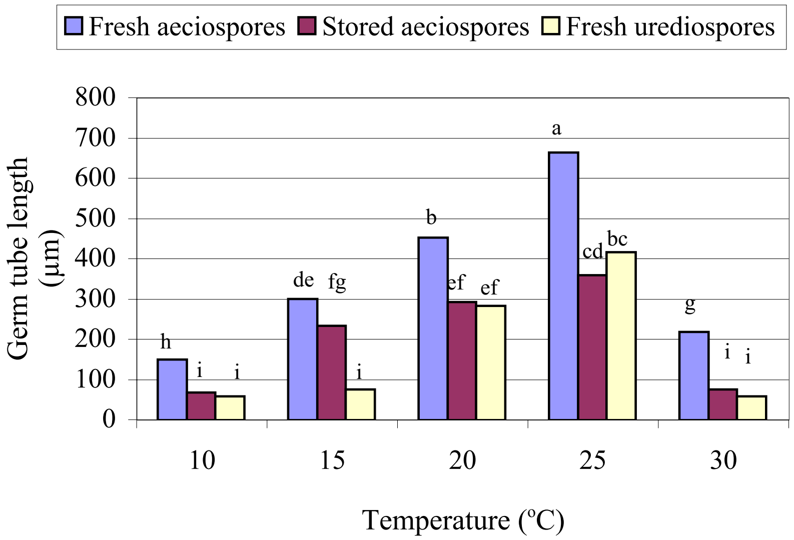

Fig. 2. Effect of temperature on germ tube length of fresh and stored aeciospores and of fresh urediospores of *Pileolaria terebinthi*. Bars with the same letter(s) are not significantly different (P=0.01).

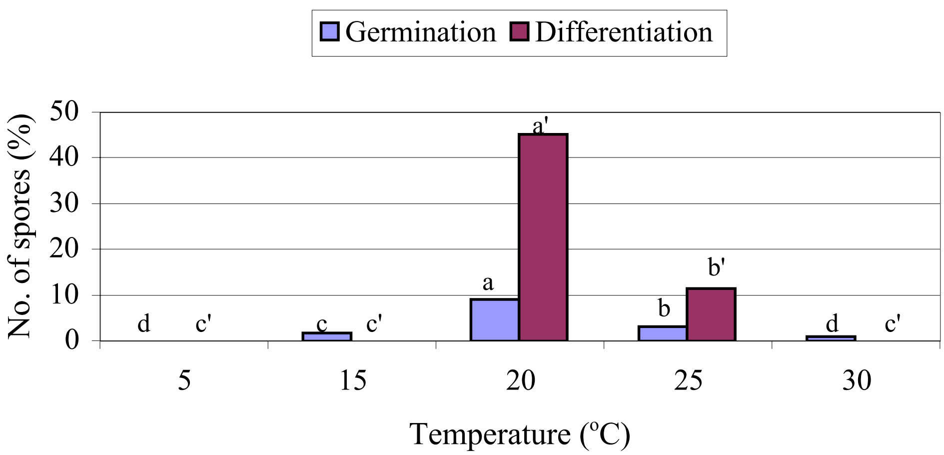

Fig. 3. Effect of temperature on teliospore germination and basidiospore differentiation of *Pileolaria terebinthi*. Bars with the same letter(s) are not significantly different (P=0.01).

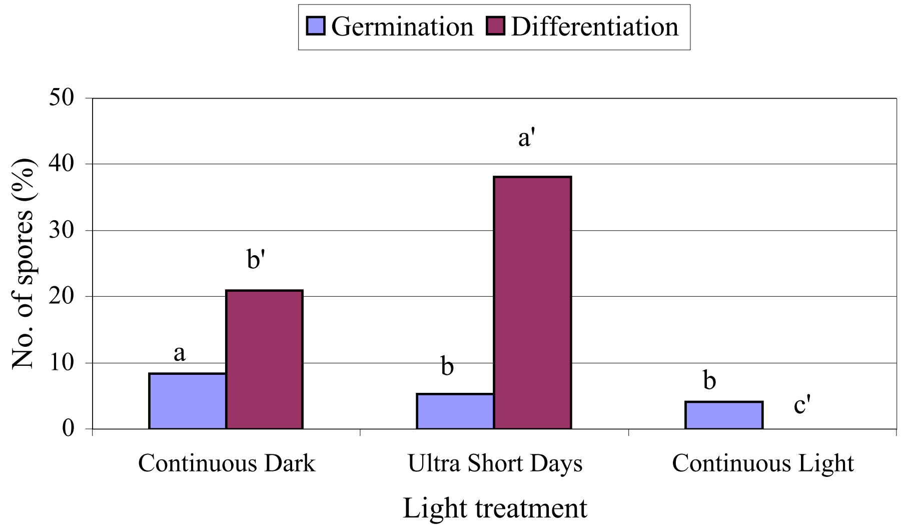

Fig. 4. Effect of light on the ratio of teliospore germination and basidium differentiation of *Pileolaria terebinthi*. Bars with the same letter(s) are not significantly different (*P*=0.01).

light treatment for teliospore germination was continuous dark, but the maximum differentiation of basidia was observed in short day exposure (23.5 h dark/0.5 h light). No basidia differentiated under continuous fluorescent illumination (Fig. 4). All teliospores samples lost their germinability after exposure for one-year outdoors.

Discussion

Favourable environmental conditions, especially moisture and temperature, are essential for epidemic outbreaks. Determining at least the relation between infection rate and temperature is essential in order to construct a forecasting model for a rust.

Rusts require sufficient free moisture on the plant surface and adequate temperatures to germinate and infect their hosts (Stubbs *et al.,* 1986). In *Pileolaria terebinthi* the range of temperatures for germination of aeciospores and urediospores were 10–30°C and 25–30°C respectively. Although germination was still high at 30°C, germ tubes produced by both spore types remained very short and sometimes became forked and abnormal (wrinkled at the apex). At the optimum temperature (25°C) and free moisture *P. terebinthi* urediospores germinate in only 4 hours, which is a very short time if we consider that, for example, the urediospores of *Tranzschelia pruni spinosa* germinate in 12 h at 22°C (the optimum temperature) and free moisture. At the optimum temperature for germination, germ tube growth rates of *P. terebinthi* urediospores and aeciospores were 17.3 μm s^{-1} and 27.62 μm s^{-1}, respectively. The lower growth rate of urediospore germ tubes may indicate a weak potential of this type of inoculum for developing an epidemic even under optimum conditions.

P. terebinthi, like many other *Uredinales*, has teliospores producing primary inoculum as basidiospores, and this may sophisticate the epidemiological forecasting models. Teliospores and basidia are essential phases in the life cycle of rust fungi. In almost half the rust species known, teliospores are dormant and require a post-ripening period to germinate (Cummins and Hiratsuka, 1983). This enables them to survive under adverse environmental conditions. Teliospores of *P. terebinthi* germinated well in the range of 15–30°C, but maximum basidium differentiation occurred at 20°C. Undifferentiated, hypha-like and malformed basidia formed at temperatures below 25°C and above 30°C. Similar observations were reported for teliospores of *Uromyces appendiculatus* var. *appendiculatus* (Gold and Mendgen, 1983a) and *Gymnosporangium juniperi-virginiana* (Aldwinkle *et al.,* 1980).

The effect of light on teliospore germination has been previously investigated and three groups have been distinguished: teliospores of the first group germinate equally well in continuous light and darkness; teliospores of the second group germinate in continuous darkness but germination is inhibited by light; teliospores of the third group need an alternation of light and darkness for successful germination (Gold and Mendgen, 1983b). Our findings indicate that *P. terebinthi* belongs to the third group.

Acknowledgements

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